

WHAT IS CLAIMED IS:

1. A method of initiating conifer embryogenic cultures comprising culturing explants using a media supplemented with biotin.
2. A culture media for initiating conifer embryogenic cultures supplemented with biotin.
3. The method of claim 1 wherein the media is supplemented with from 0.001 to 10 ppm biotin.
4. The method of claim 1 wherein the media is supplemented with about 0.001 to 1.0 ppm biotin.
5. The method of claim 1 wherein the media is supplemented with about 1.0 to 10 ppm biotin.
6. A method of initiating conifer embryogenic cultures comprising culturing explants using a media supplemented with folic acid.
7. A culture media for initiating conifer embryogenic cultures supplemented with folic acid.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

8. The method of claim 6 wherein the media is supplemented with from 0.01 to 100 ppm folic acid.

9. The method of claim 6 wherein the media is supplemented with from 0.01 to 1.0 ppm folic acid.

10. The method of claim 6 wherein the media is supplemented with about 1.0 to 10 ppm folic acid.

11. The method of claim 6 wherein the media is supplemented with about 10 to 100 ppm folic acid.

12. A method of initiating conifer embryogenic cultures comprising culturing explants using a media; and
maintaining the pH of the media at a desirable pH for the initiation of embryogenic cultures.

13. The method of claim 12 wherein the desirable pH is between 4.5 and 6.

14. A method of initiating conifer embryogenic cultures comprising culturing explants using a media supplemented with a buffer suitable for maintaining a pH of 4.5-6.0.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

2007-06-20 15:59:20

15. A culture media for initiating embryogenic cultures supplemented with a buffer suitable for maintaining a pH of 4.5-6.0

16. The method of claim 14 wherein the buffer is MES.

17. The media of claim 15 wherein the buffer is MES.

18. The method of claim 16 wherein the concentration of MES is 10 to 1000 mg/l.

19. The method of claim 16 wherein the concentration of MES is 100 to 300 mg/l.

20. A method of initiating conifer embryogenic cultures comprising culturing explants using a media supplemented with one or more gibberellin inhibitors.

21. A culture media for initiating embryogenic cultures supplemented with one or more gibberellin inhibitors.

22. The method of claim 20 wherein one or more gibberellin inhibitors are present in the initiation media at a concentration of 0.01 to 10 ppm.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

23. The method of claim 20 wherein the gibberellin inhibitor is paclobutrazol.

24. The media of claim 21 wherein the gibberellin inhibitor is paclobutrazol.

25. The method of claim 23 wherein paclobutrazol is present in the initiation media at a concentration of 0.01 to 1.0 ppm.

26. The method of claim 23 wherein paclobutrazol is present in the initiation media at a concentration of 1.0 to 10 ppm.

27. A method of initiating conifer embryogenic cultures comprising:
the application of a solution containing a gibberellin inhibitor to explants prior to culturing; and
the subsequent culturing of the explant on or in a growth media.

28. The method of claim 27 wherein the gibberellin inhibitor is paclobutrazol.

29. A method of initiating conifer embryogenic cultures comprising culturing explants in a closed container wherein the free exchange of gases with the ambient atmosphere is fully prevented.

200706533-021002

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

30. A method of initiating conifer embryogenic cultures comprising culturing explants in a closed container wherein the free exchange of gases with the ambient atmosphere is selectively reduced.

31. A method of growing previously initiated conifer embryogenic tissues, comprising growing such tissues using a media supplemented with biotin.

32. A media for growing previously initiated conifer embryogenic tissues supplemented with biotin.

33. The method of claim 32 wherein the media is supplemented with from 0.001 to 10 ppm biotin.

34. The method of claim 31 wherein the media is supplemented with about 0.001 to 1.0 ppm biotin.

35. The method of claim 31 wherein the media is supplemented with about 1.0 to 10 ppm biotin.

36. A method of growing previously initiated conifer embryogenic tissues, comprising growing such tissues using a media supplemented with folic acid.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

37. A media for growing previously initiated conifer embryogenic tissues supplemented with folic acid.

38. The method of claim 36 wherein the media is supplemented with from 0.01 to 10 ppm folic acid.

39. The method of claim 36 wherein the media is supplemented with from 0.01 to 1.0 ppm folic acid.

40. The method of claim 36 wherein the media is supplemented with about 1.0 to 10 ppm folic acid.

41. A method of growing previously initiated conifer embryogenic tissues wherein the pH of the media is maintained at a desirable pH for the growth of embryogenic tissues.

42. The method of claim 41 wherein the desirable media pH is between 4.5 and 6.

43. A method of growing previously initiated conifer embryogenic tissues, comprising growing such tissues using a media supplemented with a buffer suitable for maintaining a pH between 4.5 and 6.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

44. A media for growing previously initiated conifer embryogenic tissues supplemented with a buffer suitable for maintaining a pH between 4.5 and 6.

45. The method of claim 43 wherein the buffer is MES.

46. The method of claim 45 wherein the concentration of MES is 10 to 1000 mg/l.

47. The method of claim 45 wherein the concentration of MES is 100 to 300 mg/l.

48. A method of growing previously initiated conifer embryogenic tissues, comprising growing such tissues using a media supplemented with one or more gibberellin inhibitors.

49. A media for growing previously initiated conifer embryogenic tissues supplemented with one or more gibberellin inhibitors.

50. The method of claim 48 wherein one or more gibberellin inhibitors are present in the media at a concentration of 0.01 to 10 ppm.

51. The method of claim 48 wherein one of the gibberellin inhibitors is paclobutrazol.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

52. The method of claim 51 wherein paclobutrazol is present in the media at a concentration of 0.01 to 10 ppm.

53. The method of claim 51 wherein paclobutrazol is present in the media at a concentration of 0.01 to 1.0 ppm.

54. The method of claim 51 wherein paclobutrazol is present in the media at a concentration of 1.0 to 10 ppm.

55. A method of growing a previously initiated conifer embryogenic culture comprising culturing such tissues in a closed container wherein the free exchange of gases with the ambient atmosphere is fully prevented.

56. A method of growing a previously initiated conifer embryogenic culture comprising culturing such tissues in a closed container wherein the free exchange of gases with the ambient atmosphere is selectively reduced.

57. A method of growing a previously initiated conifer embryogenic culture wherein the atmospheric pressure of the culture vessel is maintained above 1 atmosphere for the majority of the culturing period.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

58. The method of claim 57 wherein the pressure in the culture vessel is about 1.1 to 2 atmospheres.

59. A method of improving culture capture in conifer tissue, comprising growing new initiates using a media supplemented with abscisic acid.

60. A media for improving culture capture in conifer tissue supplemented with abscisic acid.

61. The method of claim 59 wherein the abscisic acid is present in a concentration between 0.1 to 100 mg/l.

62. The method of claim 59 wherein the abscisic acid is present in a concentration between 0.1 to 1.0 mg/l.

63. The method of claim 59 wherein the abscisic acid is present in a concentration between 1.0 to 10 mg/l.

64. The method of claim 59 wherein the abscisic acid is present in a concentration between 10 to 100 mg/l.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

10076633.021902

65. The method of claim 31 further comprising:
growing the embryogenic tissue until the tissues increase in mass; and
transferring the enlarged tissues to a liquid multiplication media.

66. The method of claim 65 wherein the tissue attains a mass of at least
100 mg prior to being transferred to a liquid multiplication media.

67. The method of claim 65 wherein the tissue attains a mass of at least
200 mg prior to being transferred to a liquid multiplication media.

68. The method of claim 36 further comprising:
growing the embryogenic tissue until the tissues increase in mass; and
transferring the enlarged tissues to a liquid multiplication media.

69. The method of claim 68 wherein the tissue attains a mass of at least
100 mg prior to being transferred to a liquid multiplication media.

70. The method of claim 68 wherein the tissue attains a mass of at least
200 mg prior to being transferred to a liquid multiplication media.

71. The method of claim 41 further comprising:
growing the embryogenic tissue until the tissues increase in mass; and
transferring the enlarged tissues to a liquid multiplication media.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

72. The method of claim 71 wherein the tissue attains a mass of at least 100 mg prior to being transferred to a liquid multiplication media.

73. The method of claim 71 wherein the tissue attains a mass of at least 200 mg prior to being transferred to a liquid multiplication media.

74. The method of claim 45 further comprising:
growing the embryogenic tissue until the tissues increase in mass; and
transferring the enlarged tissues to a liquid multiplication media.

75. The method of claim 74 wherein the tissue attains a mass of at least 100 mg prior to being transferred to a liquid multiplication media.

76. The method of claim 74 wherein the tissue attains a mass of at least 200 mg prior to being transferred to a liquid multiplication media.

77. The method of claim 48 further comprising:
growing the embryogenic tissue until the tissues increase in mass; and
transferring the enlarged tissues to a liquid multiplication media.

78. The method of claim 77 wherein the tissue attains a mass of at least 100 mg prior to being transferred to a liquid multiplication media.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

10076593.021902

79. The method of claim 77 wherein the tissue attains a mass of at least 200 mg prior to being transferred to a liquid multiplication media.

80. The method of claim 51 further comprising:
growing the embryogenic tissue until the tissues increase in mass; and
transferring the enlarged tissues to a liquid multiplication media.

81. The method of claim 80 wherein the tissue attains a mass of at least 100 mg prior to being transferred to a liquid multiplication media.

82. The method of claim 80 wherein the tissue attains a mass of at least 200 mg prior to being transferred to a liquid multiplication media.

83. The method of claim 55 further comprising:
growing the embryogenic tissue until the tissues increase in mass; and
transferring the enlarged tissues to a liquid multiplication media.

84. The method of claim 83 wherein the tissue attains a mass of at least 100 mg prior to being transferred to a liquid multiplication media.

85. The method of claim 83 wherein the tissue attains a mass of at least 200 mg prior to being transferred to a liquid multiplication media.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

2007663-02100

86. The method of claim 56 further comprising:
growing the embryogenic tissue until the tissues increase in mass; and
transferring the enlarged tissues to a liquid multiplication media.

87. The method of claim 86 wherein the tissue attains a mass of at least
100 mg prior to being transferred to a liquid multiplication media.

88. The method of claim 86 wherein the tissue attains a mass of at least
200 mg prior to being transferred to a liquid multiplication media.

89. The method of claim 57 further comprising:
growing the embryogenic tissue until the tissues increase in mass; and
transferring the enlarged tissues to a liquid multiplication media.

90. The method of claim 89 wherein the tissue attains a mass of at least
100 mg prior to being transferred to a liquid multiplication media.

91. The method of claim 89 wherein the tissue attains a mass of at least
200 mg prior to being transferred to a liquid multiplication media.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

92. The method of claim 59 further comprising:
growing the embryogenic tissue until the tissues increase in mass; and
transferring the enlarged tissues to a liquid multiplication media.

93. The method of claim 92 wherein the tissue attains a mass of at least
100 mg prior to being transferred to a liquid multiplication media.

94. The method of claim 92 wherein the tissue attains a mass of at least
200 mg prior to being transferred to a liquid multiplication media.

10076533-021002

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com